Tissue Microarrays: Testing The Limits Of Paraffin Embedded Tissue

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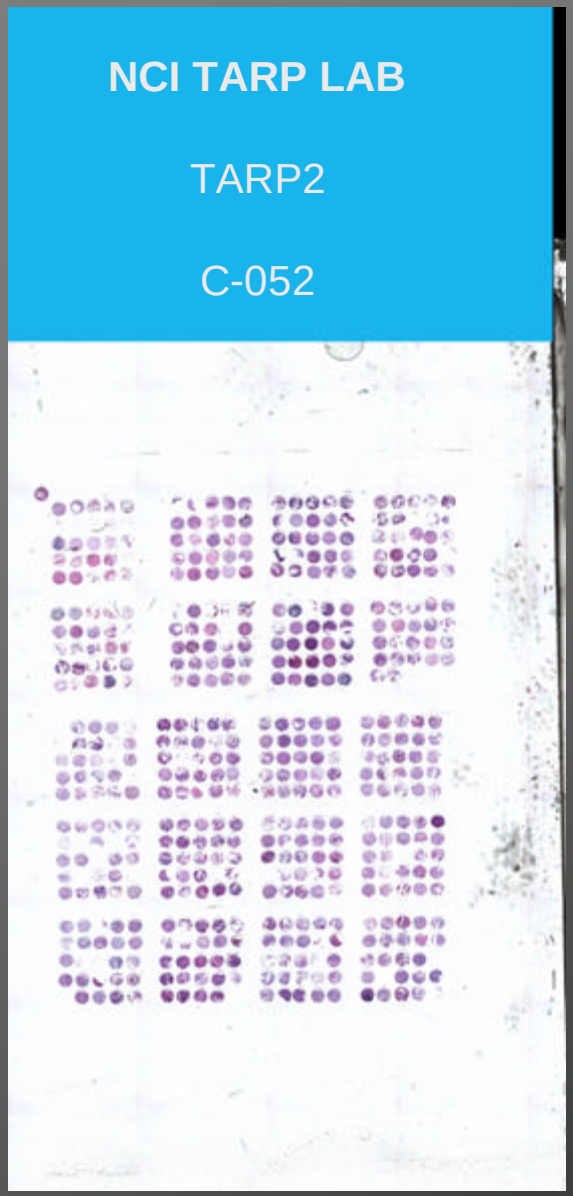
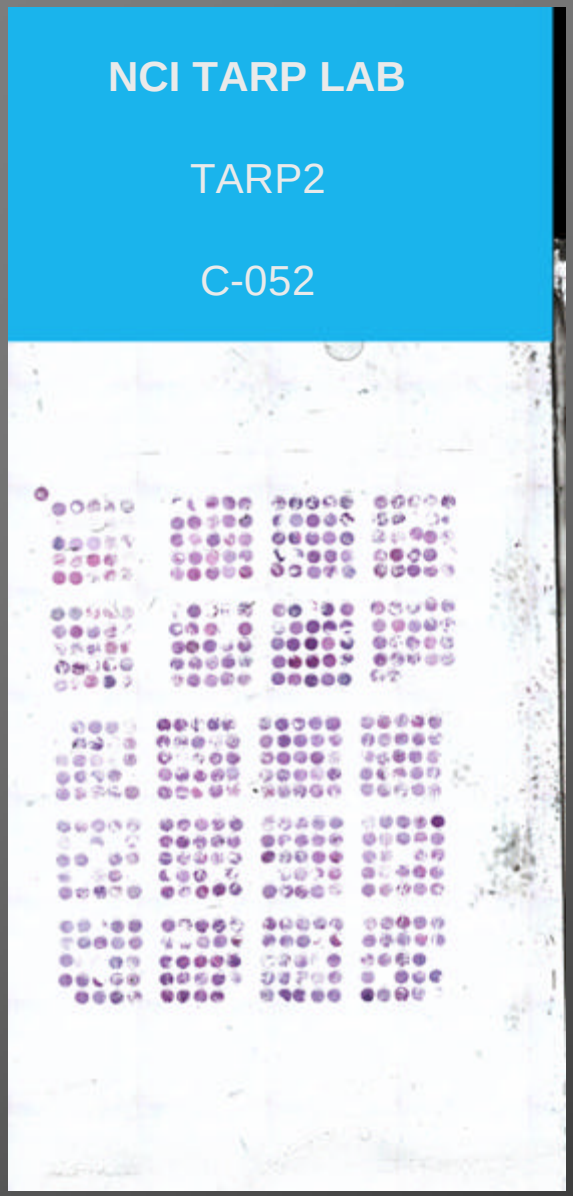
What Is A Tissue Array?



- *A Block Of Samples From Hundreds Of Blocks*
  - Multiple Samples
  - Paraffin Embedded *Or* Frozen Tissue
  - Arranged In An Organized Fashion
- *Platform For High-Throughput Pathology*

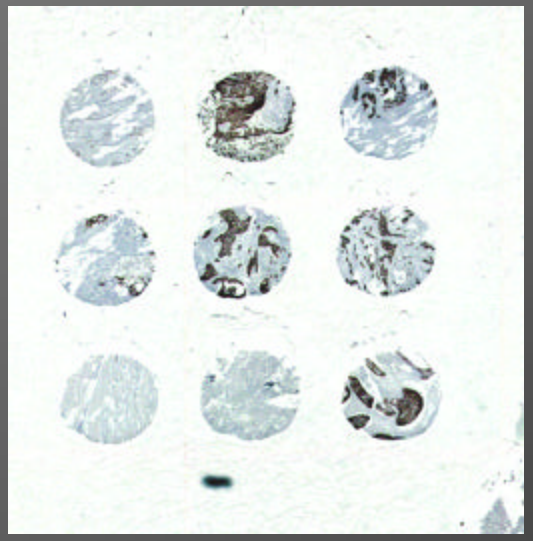
What Is A Tissue Array?

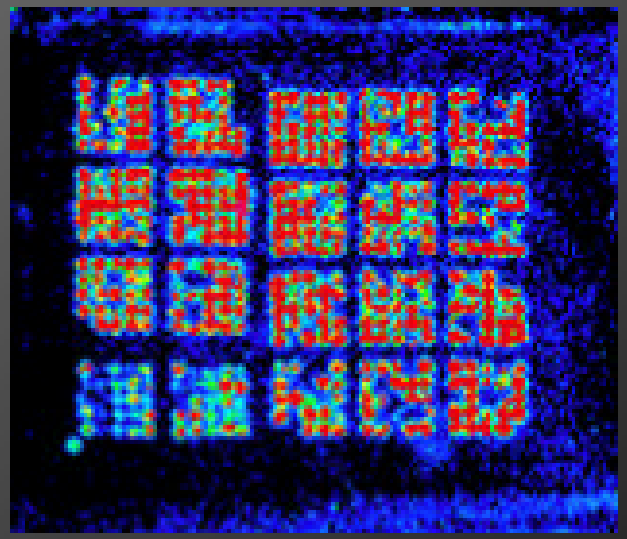


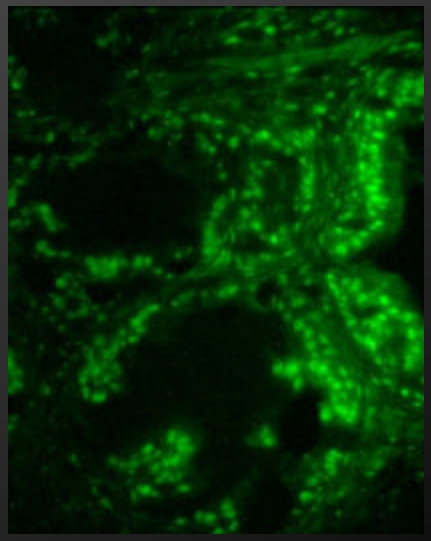
- *A Protein Array With Retained Histomorphology*
  - Cell Type Localization
  - Cell Type Quantification
  - Subcellular Localization
- *Platform For High-Throughput Proteomics*

Uses Of Tissue Microarrays

- Immunohistochemistry 95%
- *In Situ* Hybridization 4%
- Other 1%







Immunohistochemistry

Strengths

- Histomorphology
- Cytomorphology
- Simple
- Robust
- In The Clinic
  - No Specialized Equipment
  - Relatively Cheap

Weaknesses

- Quantitation
- Standards
- FFPE Tissue

Solutions

- Standards
  - Tissue Collection
  - Assay Performance
- Image Analysis

Quality Is Everything

- Quality Remains Subjective

- Tissue Quality
  - Histology
  - Proteins
  - Nucleic Acids
- Clinical Data
  - Complete
  - Detailed

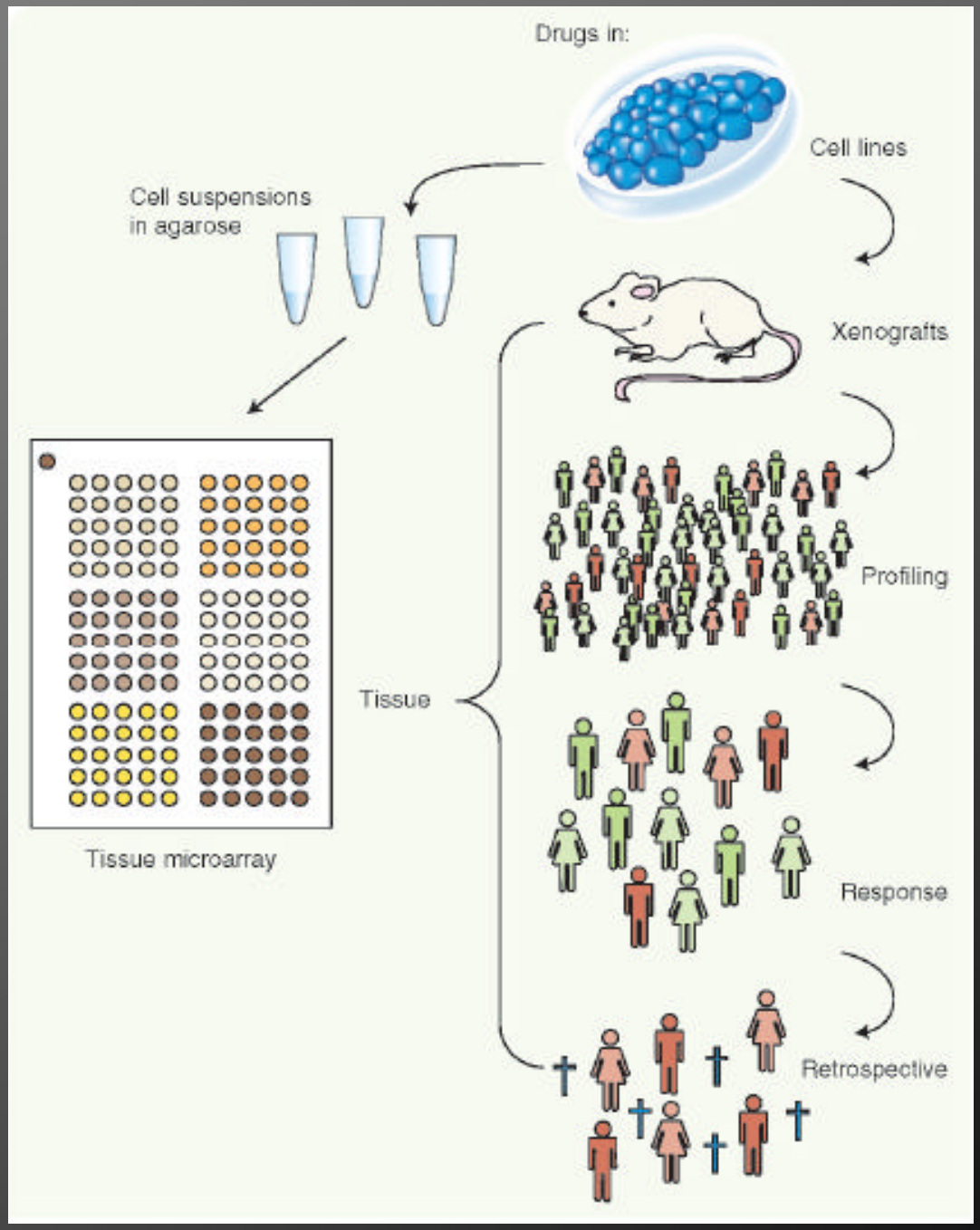
Multifaceted Role Of TMAs In Translational Research

CMAs

XMAs

TMAs

Multiple Cohorts

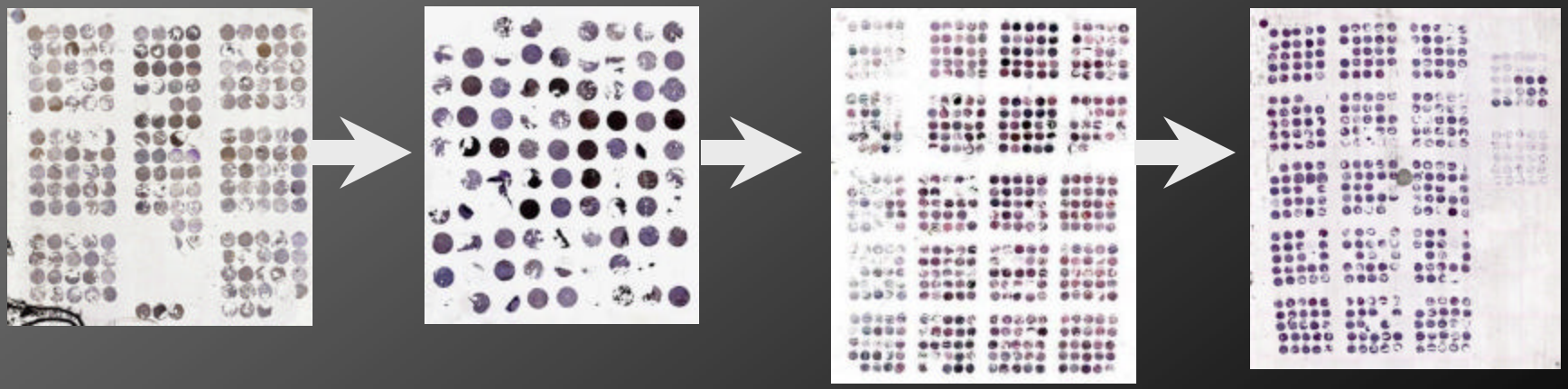


Control Of Specimen

TMAs - Tissue Microarrays

- Validate Data From Other Platforms
- Describe Expression Patterns
- Pathway Analysis
- Biomarker Development
- Model Systems –Animal Models
  - Dog Comparative Array

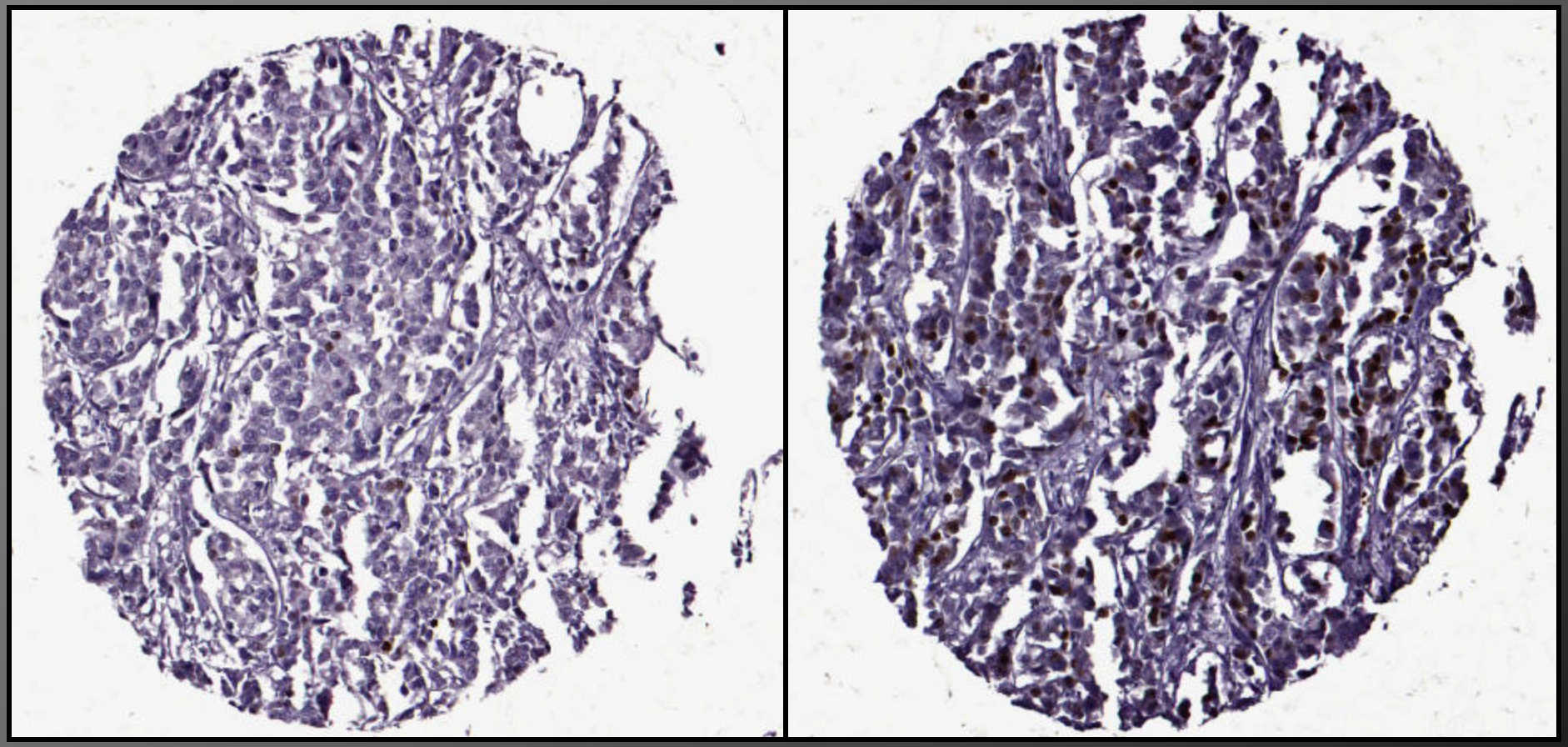
Ezrin



Challenges In The Construction Of A TMA

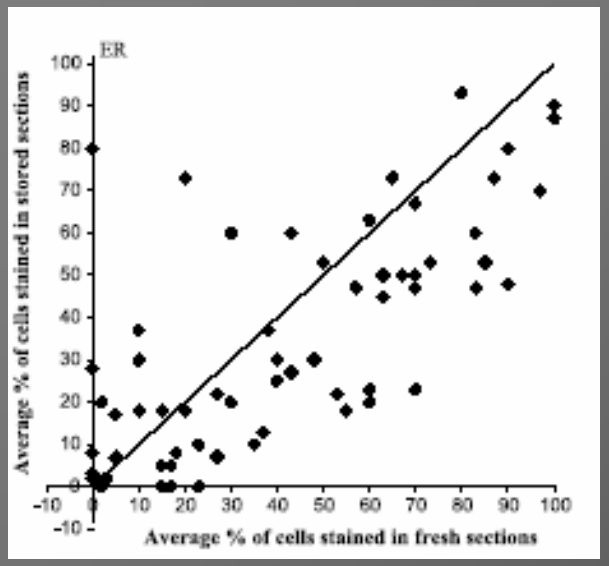
- Identifying Adequate/Appropriate Material
- Negotiating MTA/IRB Approval
- Specimens Present Special Challenges
  - Biopsy & Small Specimens Are Hard To Array
  - Prior Use Of Material For Research
- Diversity Of Specimen Sources
  - Regional Differences In Diagnosis & Treatment
  - Differences In Specimen Handling
- Informatics
- Managing Expectations
  - Pathologist Vs Scientist

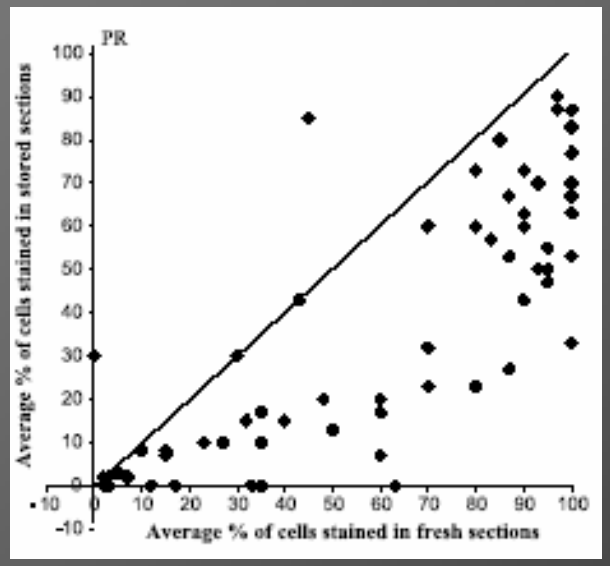
Slide Oxidation

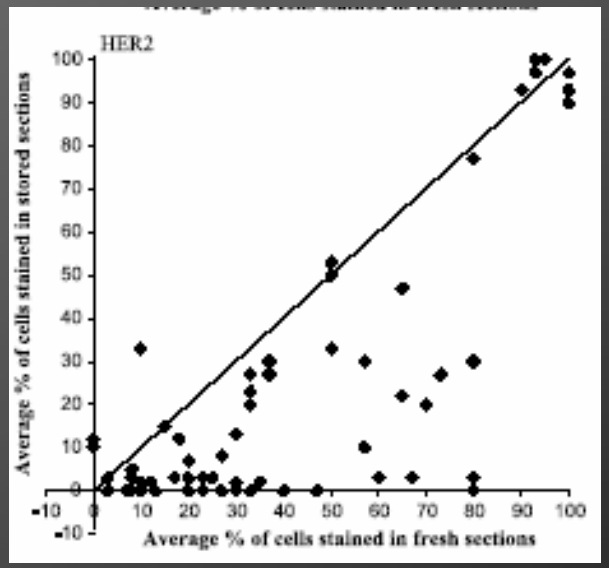


Same Core Of Two Different Slides Of The Same TMA Cut 6 Months Apart Stained At The Same Time For Progesterone Receptor

Slide Oxidation On Three Different Common Markers







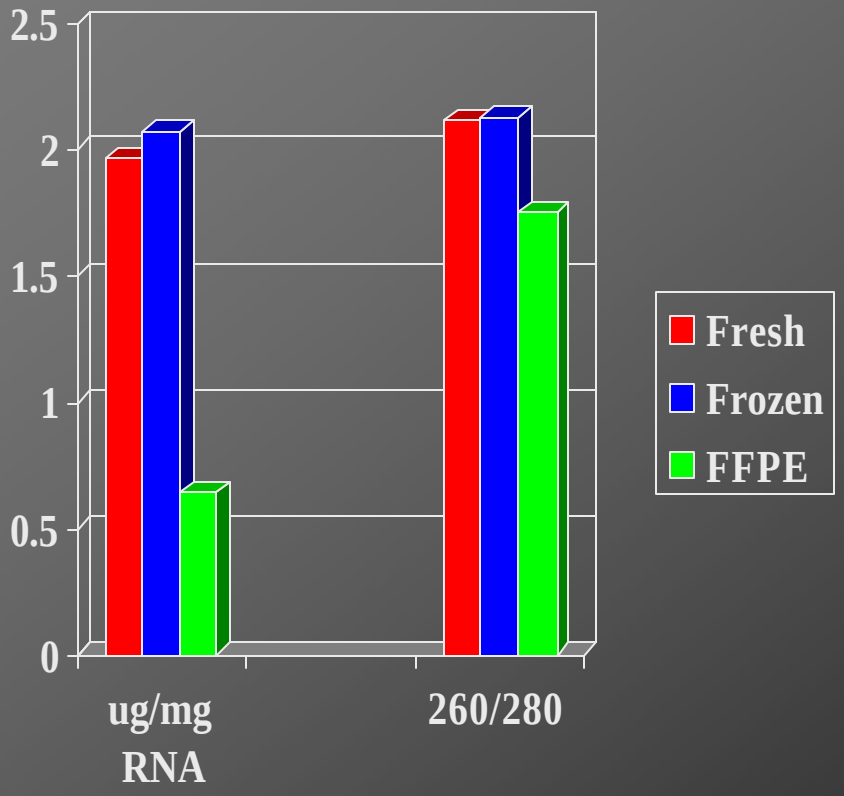
- Mechanism Of Oxidation Is Uncertain
- Tissue Handling & Processing Impacts Risk Of Oxidation
- Means Of Preventing Oxidation Are Unresolved
- Impact Of Oxidation Is Variable

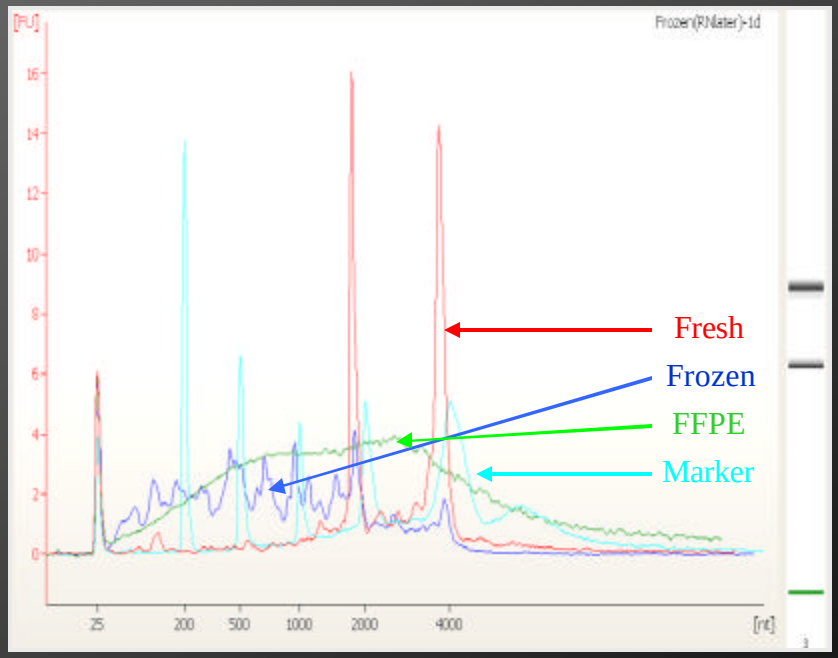
Uses Of Cores Obtained From Tissue Blocks

- Nucleic Acids
  - Expression Microarrays
  - CGH
  - LOH
- Proteins
  - Protein Arrays
  - Mass Spectroscopy
- RNA
  - 125 nt Modal
  - RT-PCR Up To 300 nt
  - 15% Of Blocks Unusable
- DNA
  - 300 bp Easy
  - Fragments Upto 1.5 kb Feasible
- Protein
  - Work In Progress

Frozen Vs FFPE Derived RNA

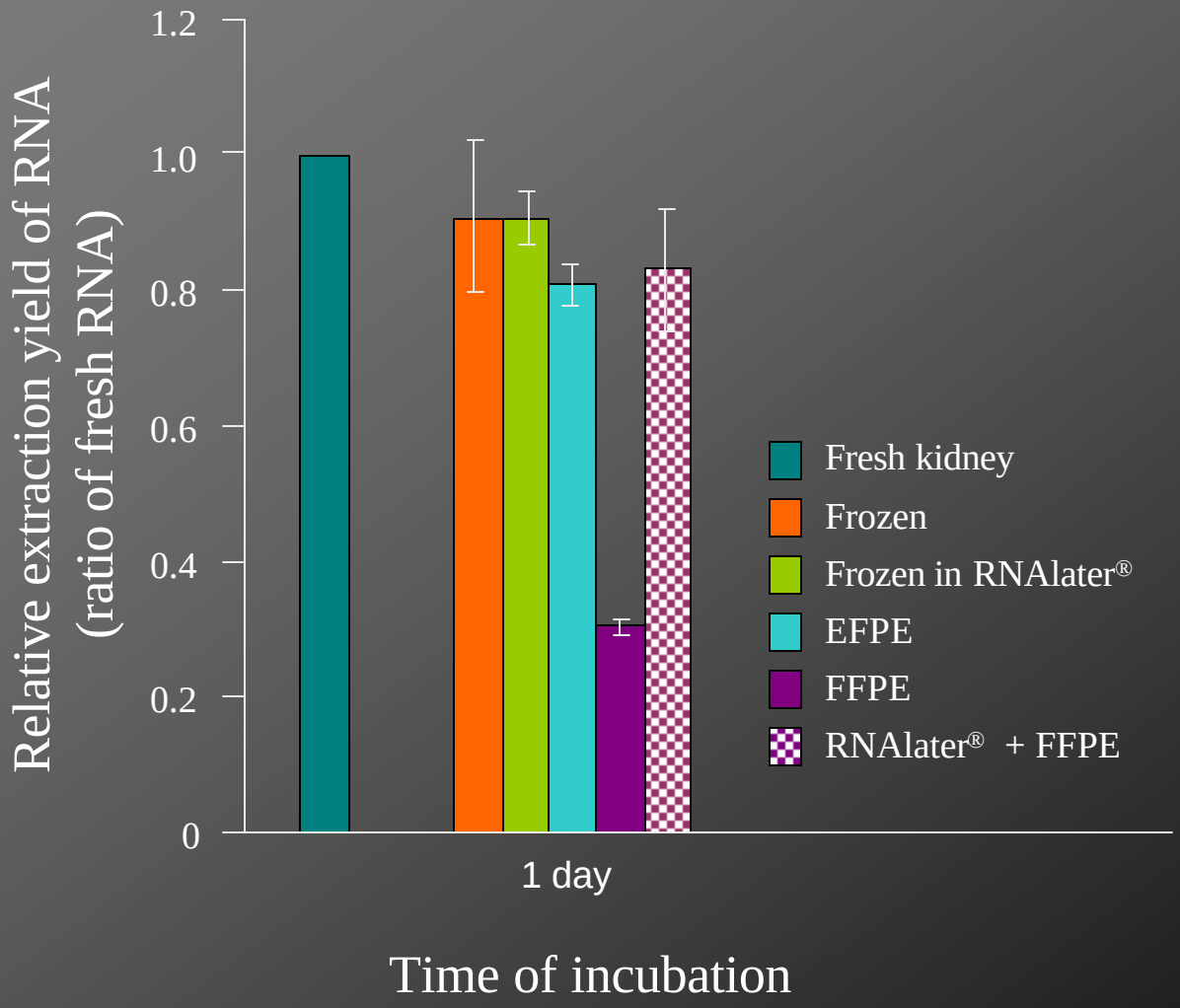
- Quantity Is Not As Decreased As Anticipated
- Quality Is Significantly Impacted



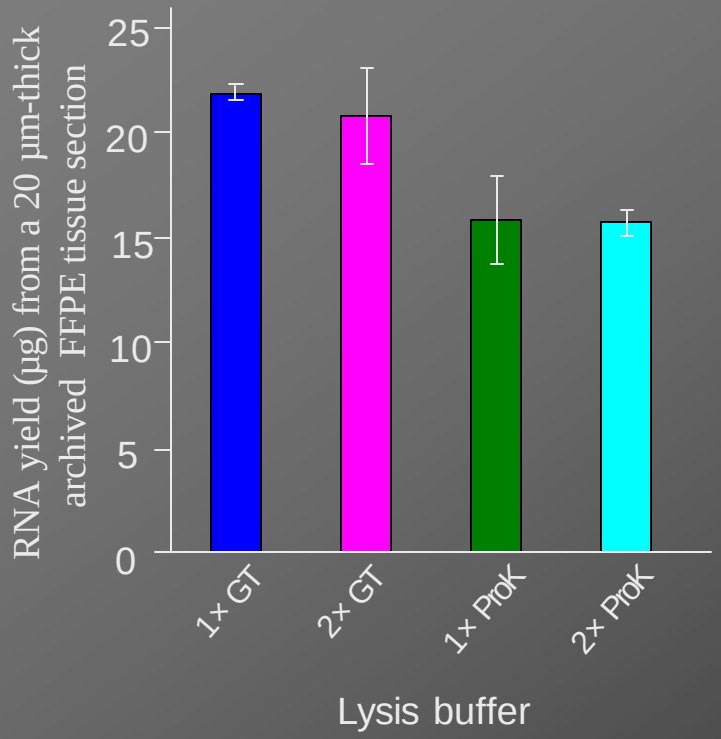


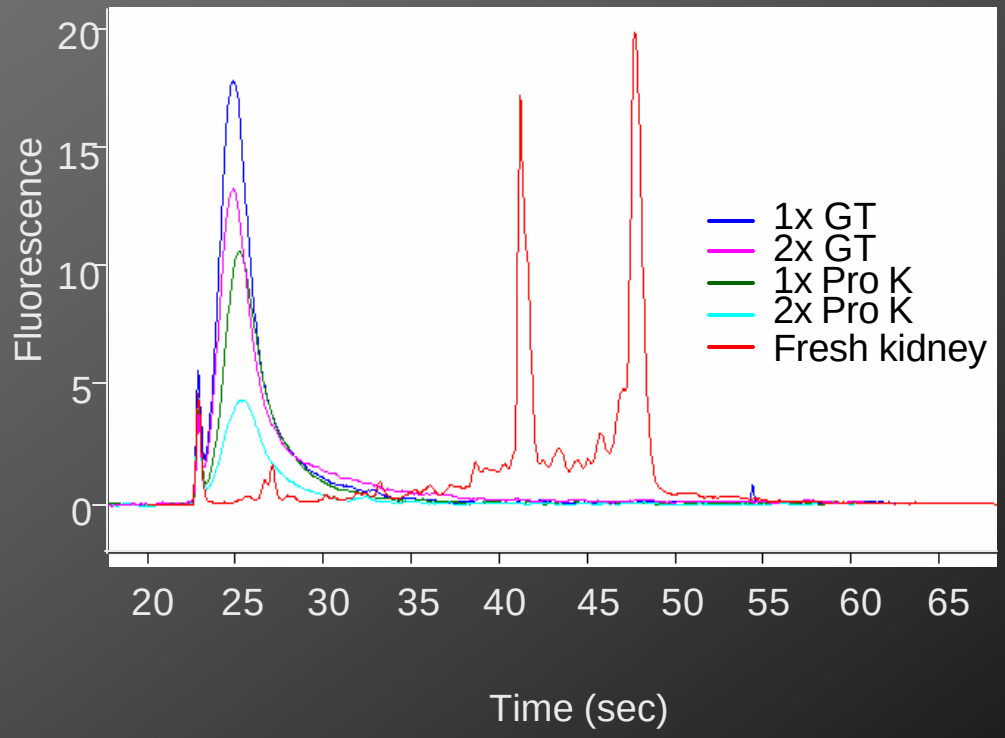
Equal Starting Material Mass Evaluation

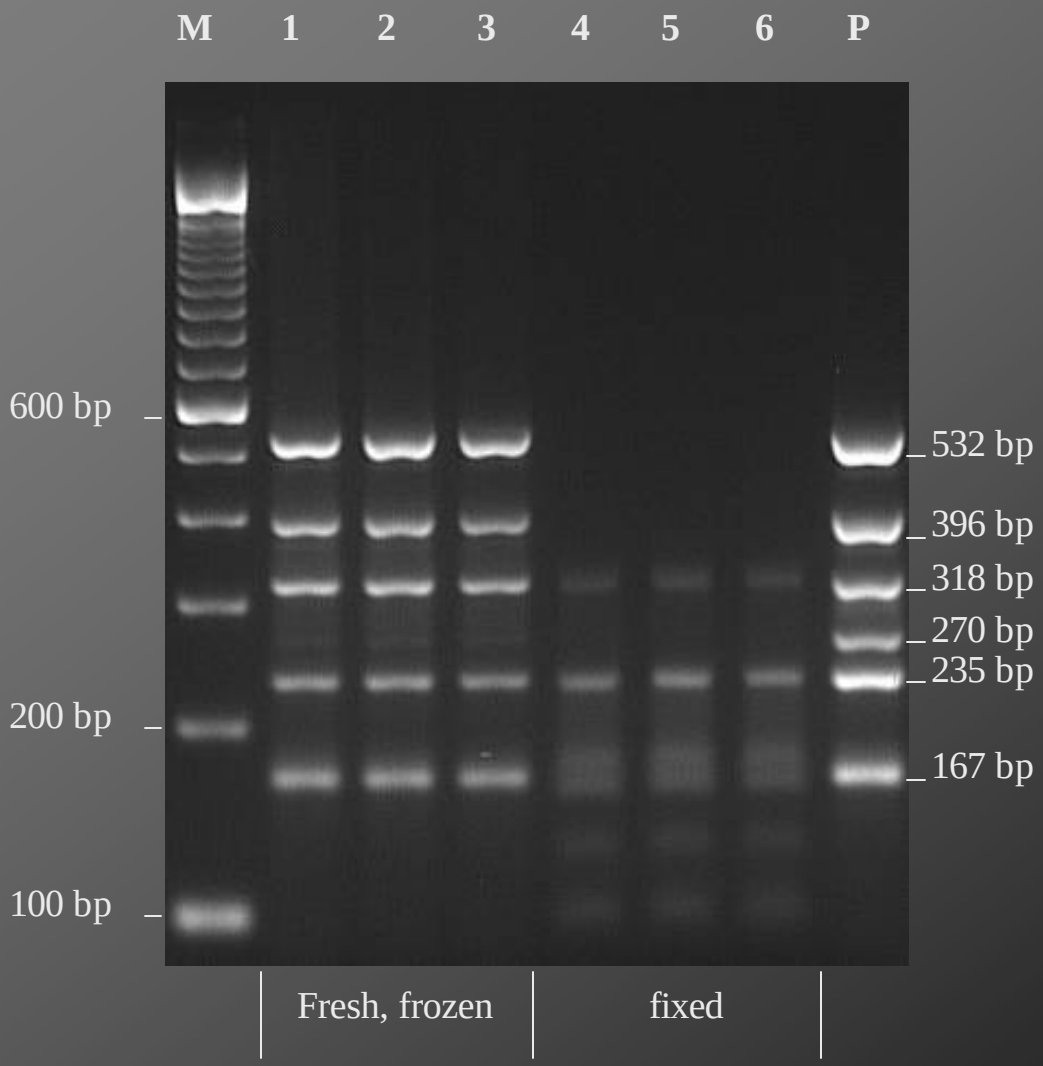
Quantitative RNA Recovery



RNA Recovery By Method






M
1
2
3
4
5
6
P
600 bp
200 bp
100 bp
532 bp
396 bp
318 bp
270 bp
235 bp
167 bp
Fresh, frozen
fixed

Tissue Preservation & Processing

Uncharacterized

- Warm Ischemia Time
- Poor Fixation
  - Inadequate Volume
  - Inadequate Time
- Poor Processing
  - Inadequate Dehydration
- Paraffin Quality
  - Hard Paraffins
  - Contaminants

Unknown

- Metabolic Effects
- Impact Of Buffers
  - What Is The Buffer?
- Alternative Processing
  - Microwave
  - Reagent Substitutions
- Storage
  - Blocks
  - Slides

The Challenges Of Tissue

Finding Tissue

Permission To Obtain Tissue

Obtaining Tissue

Preserving Tissue

Storing Tissue

Reducing Tissue To A Reagent

Performing Assays On Tissue

Interpretation Of Results

TMAs & Translation Research

- High-Throughput Pathology Is Essential
- Correlation With Outcome Essential
  - Requires Follow-Up
- Greater Attention To Quality In The Collection Of Tissue

- Forward Planning For The Collection Of Tissue In Clinical Trial Settings